PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number:
C12N 9/98, C11D 3/386	A1	WO 91/06638
		(43) International Publication Date: 16 May 1991 (16.05.91)

(21) International Application Number: PCT/US90/06290

(22) International Filing Date: 30 October 1990 (30.10.90)

(30) Priority data:
429,881 31 October 1989 (31.10.89) US

(71) Applicant: GENENCOR INTERNATIONAL, INC. [US/US]; 180 Kimball Way, San Francisco, CA 94080 (US).

(72) Inventor: BECKER, Nathaniel, Todd ; 2116 Hillside Drive, Burlingame, CA 94010 (US).

(74) Agent: PASSE, James, G.; Genencor International, Inc., 180 Kimball Way, So. San Francisco, CA 94080 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK, DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.

(54) Title: DUST-FREE COATED ENZYME FORMULATION

(57) Abstract

The current invention relates to substantially dust-free particles of enzymes produced from enzymes in fermentation broth having low concentration of enzyme solids typically from 0.5 to about 25 % of total solids, which solution is coated onto hydratable core particles in a fluidized-bad spray-coater and coated with a coating material.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**ES**	Spain	**MG**	Madagascar
AU	Australia	**FI**	Finland	**ML**	Mali
BB	Barbados	**FR**	France	**MR**	Mauritania
BE	Belgium	**GA**	Gabon	**MW**	Malawi
BF	Burkina Faso	**GB**	United Kingdom	**NL**	Netherlands
BG	Bulgaria	**GR**	Greece	**NO**	Norway
BJ	Benin	**HU**	Hungary	**PL**	Poland
BR	Brazil	**IT**	Italy	**RO**	Romania
CA	Canada	**JP**	Japan	**SD**	Sudan
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SE**	Sweden
CG	Congo			**SN**	Senegal
CH	Switzerland	**KR**	Republic of Korea	**SU**	Soviet Union
CI	Côte d'Ivoire	**LI**	Liechtenstein	**TD**	Chad
CM	Cameroon	**LK**	Sri Lanka	**TG**	Togo
DE	Germany	**LU**	Luxembourg	**US**	United States of America
DK	Denmark	**MC**	Monaco		

WO 91/06638 PCT/US90/06290

DUST-FREE COATED ENZYME FORMULATION

Field of the Invention

The present invention relates to a novel procedure for making dry and dust-free enzyme granules from a fermentation broth containing the enzyme.

Background of the Invention

Many commercially useful enzymes are produced by microorganisms, particularly bacteria, yeast and filamentous fungi. These enzymes are especially useful in detergent and food applications. With the advent of biotechnology and recombinant DNA techniques, other enzymes from mammalian sources are produced recombinantly in microorganisms. For example, chymosin, a bovine enzyme which is useful in the manufacture of certain types of cheeses, has been produced in a wide variety of microbial hosts. When enzymes are produced in a microbial host they are usually either secreted directly into the fermentation broth by the microorganism or released into the fermentation broth by lysing the cell. The enzyme can then be recovered from the broth in a soluble form by a number of techniques including filtration, centrifugation, membrane filtration, chromatography and the like. The dissolved enzyme can be converted to a dry form from a liquid using techniques such as precipitation, crystallization or spray-drying. A problem associated with dry enzyme preparations is that there is a high dust level associated with them, which can cause dermatologic distress to the manufacturer, consumer or any other person handling the enzyme. It has been a desire in the art to treat these dry enzymes so as to reduce the hazard of dusting. To control dusting and increase particle size, dry enzymes are often granulated by various means known by those skilled in the art.

Various enzyme formulations and processes for these preparations have been developed in an effort to alleviate the dusting problem. For example, German Patent No. 21 37 042 discloses a process in which an extrudable enzyme-containing formulation is extruded through a die onto the revolving plate of a spheronizing device to form spherical particles of the enzyme-containing formulations which are optionally coated with a material designed to prevent dusting.

In U.S. Pat. No. 4,087,368, there is disclosed an enzyme granule formulation in which rods or spheres of an enzyme in admixture with magnesium alkyl sulfate and ethylene oxide are provided.

U.S. Pat. No. 4,016,040 discloses a method for the preparation of free-flowing substantially dust-free, spherical enzyme-containing beads prepared by blending a powdered concentrate of the enzyme with a binder in molten form and spraying droplets of the blend through a spray nozzle into cool air to solidify the droplets and form the beads.

In U.S. Pat. No. 4,242,219, there is claimed a process for the preparation of enzyme-containing particles prepared by mixing the dry enzyme with a hydrophilic organic cohesive material, a building agent or a mixture regulating agent and mechanically dividing it into particles of the desired size and shape which are then coated with a water repellent material.

Another type of granular enzyme formulation is described in U.S. Pat. No. 4,009,076. This formulation is prepared by mixing the dry enzyme with a solid nonviable substance and optionally a cohesive organic material as

binder to form an enzymatically active core. An enzyme slurry containing the cohesive organic material can be sprayed onto, for example, sodium tripolyphosphate in a mixer or an enzyme powder can be mixed with the sodium tripolyphosphate and the cohesive organic material sprayed onto it with subsequent extrusion through a die. The enzyme-containing granule is sprayed with an aqueous solution containing a plasticized organic resin, then dried.

A process is describe in GDR Pat. 0 151 598 in which sodium tripolyphosphate is sprayed with an aqueous fermentation broth and agglomerated in a cyclone apparatus. The agglomerates are removed from the cyclone apparatus while still wet and placed in a mechanical blender with a drying detergent formulation and intensively mixed.

In British Pat. No. 1,483,591, there is described a process for coating water soluble or water dispersible particles, including enzyme particles, using a fluidized-bed reactor. This reference involves a dust-free coating technique for enzyme particles which have been granulated by other processes such as prilling or spheronizing.

In U.S. Patent 4,689,297 there is described a method for preparing dust-free enzyme involving dissolving or suspending dry enzyme in solution to make a slurry of at least 30% w/w of the solids enzymes, spraying it on a hydratable core and then coating it with macromolecular material.

Fermentation broths often contain substantially less enzyme solids than required in the above patent necessitating substantial concentration of the solution or preparing a dry product first. Starting from a dry enzyme still has the problems of dermatologic reactions for the handler.

It is desirable, therefore, to be able to produce a dry dust-free product directly from the fermentation broth without having to produce a dry enzyme product first or having to provide high concentrations (greater than 25%) of enzymes in solution.

Summary of the Invention

It has surprisingly been discovered that a dry dust-free enzyme particle can be produced from fermentation broth by the following method:

a) introducing a particulate, hydratable core material into a fluidized-bed spray-coater and maintaining the core particles suspended in the reaction chamber;

b) providing a fermentation broth containing from about 0.5% to about 25% w/w of the total solids therein of a water soluble or dispersible enzyme produced in the fermentation broth, 0.5% - 95.5% w/w of the total solids in the fermentation broth of fermentation broth solids and a total solids content of 10 - 40% w/w of the fermentation broth such that the broth has a viscosity of 10 to 5,000 cps at room temperature; and

c) spraying the broth onto the core and evaporating the liquid to leave the solids coated on the core; and optionally the additional step of:

d) spraying a coating agent over the product of step (c) and evaporating the liquid to leave the coating agent over the solids of (c) such that the total

solids added to the core provides a total dry weight gain of 25 to 210% over the initial weight of the core. Also included within the scope of the invention are the enzyme-containing particles prepared by this process.

Detailed Description of the Invention

The method of the present invention is carried out in a fluidized-bed spray-coater. Typically, such devices comprise a fluidized-bed dryer consisting of a circular product chamber that has a porous grid on the bottom and is open on the top to be put up against a conical shaped expansion chamber of a larger diameter than the circular product chamber; a filter to collect dust and help air flow is placed at the far end of the expansion chamber and a spray nozzle is located within the chamber to apply the solution to the core. In operation, as the velocity of air passing up through the chamber is increased, a point is reached where particles resting on the porous grid are suspended in the air flow as a fluid, hence the terms "fluidization" and "fluidized-bed dryer". The particles are lifted by the upward force of the air out of the product chamber into the expansion chamber where the air expands and the upward force per unit of area is reduced. This allows the particles to fall back into the product chamber and start the cycle over.

The initial step in the method involves introducing a particulate, hydratable core material into the reaction chamber of the fluidized-bed dryer and suspending the particles therein on a stream of air. The core particles preferably are composed of a highly hydratable material, i.e. a material which is readily dispersible or soluble in water. The core material should

either disperse (fall apart by failure to maintain its integrity) or dissolve by going into a true solution. Clays (bentonite, kaolin), non-pareils and agglomerated potato starch are considered dispersible. Non-pareils are spherical particles consisting of a solid core that has been rounded into a spherical shape by binding layers of powder to the core in a rotating spherical container and are preferred.

Salt particles (NaCl crystals, NaCl rock salt, $NaHCO_3$) are considered soluble. More particularly, core particles can be non-pareils with or without a final coat of dextrin or a confectionery glaze. Also suitable are agglomerated trisodium citrate, pan crystallized NaCl flakes, bentonite granules and prills, bentonite/kaolin/diatomaceous earth disk-pelletized granules and sodium citrate crystals. The core particle is of a material which is not dissolved during the subsequent spraying process and is preferably of a particle size from 150 to 2,000 microns (100 mesh to 10 mesh on the U.S. Standard Sieve Series) in its longest dimension.

By fermentation broth is meant the liquid in which the enzyme is produced by fermentation in a microorganism. The broth may be modified by deleting or adding material, e.g., filtration of cell solids or addition of binders, salts, pigments, binders, plasticizers and fragrances, however, it still contains the enzyme. It also may be concentrated by removal of a portion of the liquid material.

Enzymes suitable for use in this method are those which are soluble or dispersible in the fermentation broth they are produced in and from which the volatile components of the fermentation broth can be removed to leave a

residual layer of enzyme on the surface of the core material. Suitable enzymes include, for example, proteases (bacterial, fungal, acid, neutral or alkaline), amylases (alpha and beta) and lipases preferred enzymes include subtilisins and cellulases which have not been separated from their fermentation broth. The enzyme is present in the broth at from about 0.5% to about 25% w/w of total solids, and fermentation broth solids range from about 0.5% to about 95.5% w/w of total solids in the fermentation broth, with any remaining solids comprising added metallic salts, pigments, binders, plasticizers and fragrances such that total solids represent 10 - 40% w/w of the fermentation broth. The broth, including any optional metallic salts, pigments, binders, plasticizers or fragrances, must have a viscosity low enough (typically 10 to 5,000 cps at room temperature) to be pumped and atomized for effective spray-coating. The broth solids and enzymes are applied to the surface of the core material by fluidizing the core particles in a flow of air whereupon a broth containing the enzyme and other solids is then atomized and sprayed into the expansion chamber of the spray-coater. The atomized droplets contact the surface of the core particles leaving a film of the solids adhering to the surface of the particles when the water and other volatiles are evaporated.

Airflow is maintained upwards and out the top of the expansion chamber through a filter. The filter may be located inside or outside of the unit, or may be substituted for by a scrubber or cyclone. This filter traps fine dried particles which contribute to dust. Fluidized-bed spray-coaters that have this filter typically have automatic shakers which shake the filter to prevent excessive restriction of the air flow. In a preferred embodiment, the shaker unit is turned off during the last 5 minutes of operations, thus

reducing the dusting due to release of fines trapped within the filter. In another preferred embodiment, the filter is located outside the unit or substituted by a scrubber or cyclone.

When recovering fermentation broth enzymes, the broth may be treated in various ways to achieve desired results. For example, the broth may be filtered to remove cells and cell debris or to remove microorganisms to yield a sterile product. The broth may be concentrated to achieve the desired total solids concentration of 10 - 40% w/w of the broth. Further, as mentioned above, salt, stabilizers, etc. can also be added as desired. It is further a preferred embodiment of the invention that the weight gain of the solids in the broth applied to the core over the initial dry weight of the core is greater than 35% up to about 185% w/w. In another preferred embodiment the weight gain is greater than 35% to about 50% w/w.

Handling the enzyme in liquid form in the fermentation broth has the advantage of lowering the possibility of dermatologic contact due to dusting and produces a product which is dust-free and minimizes losses due to any extra step of drying.

When sufficient enzyme is applied to the core particles to provide the desired enzyme activity, the enzyme coated particles, while still suspended in the reaction chamber of the coater or later reintroduced therein, are coated with a layer of a water soluble or water dispersible coating agent. This is accomplished in a manner similar to that used for application of the enzyme/solids coating. Suitable coating agents include, for example, fatty acid esters, gum arabic and other natural gums, alkoxylated alcohols,

polyvinyl alcohols, ethoxylated alkylphenols and more specifically, polyethylene glycols (MW 300 to 8,000), linear alcohol alkoxylates (MW 1,450 to 2,670), polyvinyl pyrrolidone (MW 26,000 to 33,000), cellulose acetate phthalate (CAP), polyvinyl acetate phthalate (PVAP), polymeric nonylphenyl ethoxylates (MW 1,975 to 4,315) dinonylphenyl ethoxylate (average MW 6,900) hydroxypropylmethyl cellulose and other modified celluloses. Other coating agents include sugars, starches, titanium dioxide, and other binders or pigments. The net result of the process is to provide an enzyme coated core particle having a layer of the coating agent on its surface to provide the desired dust-free enzyme-containing particle. The total weight gain of solids versus the initial core is from about 25% to about 210% preferably from about 50 to about 100% most preferably from about 55 to about 75%.

The dust-free enzyme particles of the present invention can be used wherever enzymes are needed in a dry system. Thus, they can be used as additives to dry detergent formulations, for removing gelatin coatings on photographic films to aid in silver recovery, in the digestion of wastes from food processing plants for nitrogen recovery, in denture cleansers for removing protein bound stains in food preparation and as a processing aid in waste water treatment.

The following examples are representative and not intended to be limiting. One skilled in the art could choose other enzymes, broths, cores and coating agents based on the proportions of ingredients taught herein.

Preferred Embodiments

Example 1 - Fluidized-Bed Spray Coating of Alkaline Protease (subtilisin).

884 grams of non-pareil seeds (cores), -20/+40 mesh, were placed into a Uni-Glatt laboratory fluidized-bed spray-coater, fluidized and heated to 48°C to reduce stickiness. 1910 ml of an aqueous concentrated fermentation broth of subtilisin with 19.2% w/w total dry solids as a percentage of the fermentation broth and 10.4% w/w dry enzyme solids as a percent of total solids was pumped into the fluidized-bed through a 1.2 mm nozzle at an average rate of 13.7 ml/min. A total of 339 grams of fermentation solids were added to the initial charge weight (38.3% w/w). After sieving to achieve a -16/+40 cut, 1147 grams at 3.1% w/w enzyme were recovered from the Uni-Glatt, representing a 92.4% enzyme recovery.

The enzyme-coated granules were then charged into the Uni-Glatt, fluidized and heated to 44°C. A coating solution consisting of 248 g of water, 135 g PEG 8000, (30% w/w) and 68 g TiO_2 (15% w/w). After sieving to achieve a -16/+40 cut, 1300 g of dust-free granules containing 2.5% w/w enzyme were retrieved.

Adjusting for losses in sieving, the coated granules had a net weight gain of 59.8% over the original charge of cores. The actual net recovery of active enzyme was 85.6%.

The operating conditions in the Uni-Glatt were as follows:

Spray Mode:	Top spray
Nozzle diameter:	1.2 mm.
Atomization Air Pressure:	2.5 Bar
Ventilator Air Flap Position:	25 - 35°
Inlet Air Temperature Setting:	45 - 50°C
Outlet Air Temperature Range:	43 - 49°C
Filter Shaking Interval:	2 minutes, off during last 5 minutes
Filter Shaking Duration:	7 seconds

Example 2 - Lab Scale Fluidized-Bed Spray-Coating of Alkaline Protease

A second batch of granulated alkaline protease was made by the same procedure as that described in Example 1. The key process parameters were as follows:

Initial weight of cores	=	940 grams
Total Feed Solids	=	16.0% w/w
Enzyme Percent of Feed Solids	=	10.6%
Weight Gain From Broth	=	38.3%
Weight Gain From Coating	=	15.4%
Total Weight Gain	=	61.2%
Net Enzyme Recovered	=	80.0%

Example 3 - Lab Scale Spray-Coating From High Solids Protease Broth

A 563 gram quantity of non-pareil seeds (cores), -20/+40 mesh, was added to the Uni-Glatt. A 4100 ml protease fermentation broth concentrate with 23.6% total solids and 1.17% enzyme solids (i.e. enzyme was 4.96% of total solids)

was sprayed onto the cores, resulting in 1490 grams, or an addition of 927 g in weight (165% weight gain) to the cores after sieving to -20/+40 mesh. To 1116 grams of these enzyme-coated cores, a coating solution of 131 grams PEG 8000 (MW 8000), 66 grams TiO_2, and 241 grams water was sprayed on. The final product, 1303 grams in weight, had a 79% recovery with a 209% net weight gain.

Example 4. Lab Scale Spray Coating of Alkaline Protease.

In a Uni-Glatt fluidized-bed spray-coater, 853 grams of salt-core non-pareils were fluidized and heated to 44°C. A 555 ml ultrafiltration concentrate containing 18.6% w/w total solids and 4.31 % w/w enzyme solids (i.e., 23.2% of solids were enzyme) was sprayed onto the cores, resulting in 961 grams of product (a 12.7% increase in mass). This material was harvested and screened, samples were removed, and 744 grams were recharged into the clean coater. The granules were coated with an aqueous solution of 30% w/w PEG 8000 an 15% TiO_2, resulting in 875 grams of product (a 17.6% overcoat). The total increase in weight of the cores was 32.5%.

Example 5. Lab Scale Fluidized-Bed Spray Coating of Fungal Cellulase

In a Uni-Glatt spray-coater, 1000 grams of -20/+40/salt/core non-pareils were fluidized and heated to 40°C. 550 ml of cellulase concentrate with 24.5% w/w solids, 10.9% w/w enzyme solids (44.5% of total) was sprayed on at 10 ml/min, resulting in 1140 grams product, a 14.0% w/w increase over the initial cores. No coating was added.

Example 6. Lab Scale Fluidized-Bed Spray-Coating of Bacterial Lipase

In a Uni-Glatt spray-coater, 605 grams of -20/+40 non-pareils were fluidized and heated to 40°C. 460 ml of lipase concentrate with 26.2% w/w solids, 2.26% w/w enzyme solids (8.6% of total) was sprayed on at 8 ml/min, resulting in 711 grams product, a 17.5% w/w increase over the initial cores. To 586 grams of this material, a coating of 57.5 g PEG 8000 and 28.8 g TiO_2 (in 105.4 g of water) was added, resulting in 632 grams product, a 7.8% increase in weight. The net increase in weight was 26.7% w/w.

Claims

1. A process for providing a dry dust-free particle from the fermentation broth in which the enzyme was produced, comprising:

a) introducing a particulate, hydratable core material into a fluidized-bed spray-coater and maintaining the core particles suspended in the dryer's reaction chamber;

b) providing a fermentation broth containing from about 0.5% to about 25% w/w of the total solids therein of a water soluble or dispersible enzyme produced in the fermentation broth; 0.5% - 99.5% w/w of the total solids in the fermentation broth of fermentation broth solids and a total solids content of 10 - 30% w/w of the fermentation broth such that the broth has a viscosity of 10 to 5,000 cps at room temperature; and

c) spraying the broth into the core and evaporating the liquid to leave the solids coated on the core.

2. A small process according to Claim 1 which comprises the further step of:

d) spraying a coating agent over the product of step (c) and evaporating the liquid to leave the coating agent over the solids of (c) such that the total solids added to the core provide a total dry weight gain of from 25% to about 210% over the initial weight of the core.

3. A process according to Claim 1 wherein the fermentation broth has been filtered to remove microorganism cells or cell debris.

4. A process according to Claim 1 wherein the enzyme is a chymosin.

5. A process according to Claim 3 wherein the enzyme is a subtilisin.

6. A process according to Claim 3 wherein the enzyme is a cellulase.

7. A process according to Claim 3 wherein the enzyme is a lipase.

8. A process according to Claim 1 wherein the enzyme is present at about 5 - 25% w/w of the total solids in the enzyme broth.

9. A process according to Claim 1 wherein the weight gain of the total solids in the fermentation broth over the weight of the core is greater than 35% up to about 185% w/w.

10. A process according to Claim 1 wherein the total dry weight gain of the product in d) is from about 50% to about 210% w/w.

11. A process according to Claim 1 which comprises utilizing a spray-coater with a filter located outside of the spray-coater reaction chamber.

12. A process for producing a dust-free product using a spray-coater having an automatic filter shaking device for the filter at the top

of the expansion chamber, the improvement comprising turning off said automatic shaking device during the last 5 minutes of the coating process.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 90/06290

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: C 12 N 9/98, C 11 D 3/386

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	C 12 N; C 11 D

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	EP, A2, 0193829 (MILES INC.) 10 September 1986, see the whole document	1-12
X	Patent Abstracts of Japan, Vol 10, No 366, C390, abstract of JP 61-162185, publ 1986-07-22 NAGASE SEIKAGAKU KOGYO K.K.	1-12
A	Patent Abstracts of Japan, Vol 9, No 166, C290, abstract of JP 60- 37983, publ 1985-02-27 SHOWA DENKO K.K.	1-12
A	AU, B, 24547/84 (SANDERS) 22 August 1985, see the whole document	1-12

* Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
7th February 1991	- 1. 03. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	EPO PEIS M. Pers

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	EP, A2, 0304332 (NOVO INDUSTRI A/S) 22 February 1989, see the whole document -- --------	1-12

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/US 90/06290

SA 41948

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 28/12/90
The European Patent office is in no way liable for theseparticulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A2- 0193829	10/09/86	NONE	
AU-B- 24547/84	22/08/85	NONE	
EP-A2- 0304332	22/02/89	NONE	

For more details about this annex : see Official Journal of the European patent Office, No. 12/82